STUDIES ON DERMATOPHYTES AND RELATED KERATINOPHILIC FUNGI ISOLATED FROM SOIL OF ANIMALS HABITAT AND AGRICULTURAL FIELDS OF SAHARANPUR (U.P)

A SYNOPSIS

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Introduction

Keratinophilic fungi are potentially pathogenic to humans and animals and also dermatophytes. Studies on soil inhabiting keratinophilic fungi from various habitats are receiving considerable attention in recently years **(Kushwaha *et al*., 1976).** These are a group of fungi that colonize various keratin substrates and degrade them. Their distribution is variable with the environment and depends on different factors such as human and animals. Occurring in nature mainly in the form of hairs, wools, feathers, nails and other cornfield appendages constitutes natural baits for these fungi **(Rizwana *et al*., 2012).** The biological functionof keratinolytic fungi in the soil is the degradation of keratinophilic fungi materials such as hides, furs, claws, hair and horns of death animals. The species of natural habitats anthrophilic when humans, are the host, zoophilic when a variety of animals represent the host, geophilic, soil is the natural habitat keratinophilic fungi. The occurrence of dermatophytes in soil was reported for the first time used the hair baiting technique **(Tancinova *et al*., 2013).** Keratinophilic fungi live on the hair coat of dogs and cats, most of them cannot produce damage but among them called dermatophyte sare infectious agents and cause the zoonotic disease dermatophytosisor"tinea".The clinical dermatophyte fungal infections are also referred as "ring worm these fungi are classified into three genera: *Microsporum, Trichophyton* and *Epidermophyton.* These genera include many species infection begins after physical contact with an infected outside source. Geophilic dermatophytes are found in the soil, Zoophilic on animals such as Dogs, in Anthrophilic in infected humans, dermatophytes create inflammation and invading the keratin of the stratum corneum as well as the nails and the hairs shaft (**Tapia *et al.,* 2000).** This could help us to know the distribution and occurrence of dermatophytes and other keratinophilic fungi and risk of human dermatophytosis in those regions which could have a role in degradation of keratinous material as an industrial point of view **(Mahmoudi *et al*., 2006).** Constant exposure to fungal spores in the atmosphere can induce respiratory allergies. Elevated antibodies to a range of common spore forming fungi have been demonstrated in occupational diseases such as Humidifier fever, Malt workers' lung, Farmers' Lung and Wheat throe shers' disease. Certain fungi, such as mushrooms can produce poisonous toxins that may prove fatal if ingested. Many moulds produce secondary metabolites that are highly toxic to humans **(Kumari *et al*., 2012)**. Keratinophilic fungi and leather layers **(Apta Chaitanya *et al.,* 2013).** The species composition of fungi and fungus like organisms depends on many environmental factors include physical and chemical parameters of the

water. Dighnhr .S these water organisms together with bacteria decompose the organic compounds and have an important function for the matter cycle in the water environment **(Kiziewicz *et al.,* 2004).** Keratins are insoluble proteins that are major constituents of skin, hair, feathers, wool, and nails. Compared to other soluble proteins, keratins are known for their high stability and extreme resistance to degradation by proteolytic enzymes such as pepsin, trypsin, and papain **(Luo et al., 2010)**. Enzymes are well known biocatalysts that perform a multitude of chemical reactions and are commercially exploited in the detergent, food, pharmaceutical, diagnostics, and fine chemical industries. Keratinolytic enzymes have been studied from a variety of fungi, but to a lesser extent in bacteria. With this background, this study was designed to identify feather degradin bacteria from poultry farm soil which produce high amount of keratinase extracellular enzyme used for the biodegradation of keratin **(Vigeneshwaran *et al*., 2010)**. Keratinase is produced only in the presence of keratin substrate. Keratinase attacks the disulphide bond of keratin to degrade it some microbes have been reported to produce keratinase in the presence of keratin substrate. Keratinase producing microorganisms have ability to degrade chicken feathers, hair, nails in the current study we focused on the isolation and characterization of extracellular keratinase producing bacteria from the soil of regular feather dumping site of poultry processing plant **(Agrahari *et al*., 2013).** There is need for scientific approach involving detailed chemical and fungi toxic analyses of the plant which are being used against skin diseases in empirical ways. With the introduction of modern scientific method and techniques the validity of these drugs can be ascertained in different domains of fungal pathology. It is encouraging that many botanical preparations have been found effective and have occupied an important place in modern clinical practice unfortunately scientific and systematic investigations of green plant for their antifungal activity against ringworm are scarce. Only the volatiles of plants have been screened for their antifungal activity **(Kishore *et al.,* 1992).** Onychomycoses are infections of the nails by fungi. Human infections, particularly those involving dermatophytes (*Trichophyton, Epidermophyton floccosum & Microsporum)* and non-dermatophytic fungi (*Aspergillus* & *Candida albicans*) constitute a serious problem, especially in tropical and subtropical developing countries **(Raza 1998).** Dermatophytes are keratinophilic fungi capable of causing dermatophytosis (commonly known as tinea or ringworm) in human and animals **(Liu *et al.*, 1997).** The most common species of dermatophytes were *Trichophyton*, *Microsporum* and *Epidermophyton* which cause superficial fungal infections of skin. The overuse of antibiotics in the treatment of infectious disease and the appearance of multi-drug resistant fungal strains (resistant to two or more antibiotics) accompanied with lack of

efficacy and side effects, has driven the research towards the study of antimicrobial agents from essential oils **(Cox *et al*., 2000; Dorman & Deans 2000).** Resistance to drugs as well as limiting toxic effects has stimulated the search for new groups of antimitotic agents **(Sternberg 1994).**Much attention was drawn to plant-derived fungicides, based on the knowledge that plants have their own defence against fungal pathogens **(Gurgela *et al*., 2005).** Plants are capable of sensing the presence of potential photo pathogens (fungi, bacteria & viruses) and can produce antifungal compounds to protect themselves from biotic attack that could be essential for fungal infection resistance **(Wojtaszek 1997).**In traditional medicine many plants were claimed to be effective against fungal pathogens and research on these may yield potential leads for the development of drugs effective against humans, pathogenic fungi**(Kim *et al*., 2003; Navarro *et al*., 2003; Souza *et al.*, 2003; Rajendraprasad *et al*., 2004)**. Olive oil is obtained from the fruit of olive, which contains different fatty acids, high proportion of oleic acid (65 - 85%) **(Gunstone *et al.*, 1994; Neeman 2000).** The increasing resistance to antifungal compounds and the reduced number of available drugs led us to search for the new alternatives among aromatic plants and their essential oils, used for their antifungal properties. The antifungal activity can be attributed to the presence of some components such as carvacrol, α-terpinly acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity **(Knobloch *et al*., 1985).** A number of scientific investigations have highlighted the importance and the contribution of many plant families i.e. Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae, etc., used as medicinal plants **(Sheetal *et al.*, 2008).** Infectious diseases accounts for high proportion of health problems in the developing countries including India Microorganism have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious disease has been created the resistance of the organisms Increased due to indiscriminate use of commercials antimicrobial drugs commonly used for the treatment of infectious disease **(Davies *et al.*, 1994).** This situation forced the researchers to search for new antimicrobial substance from various source including medicinal essential oil plant **(Bauer *et al.,* 1996).** Studies over the past hundred years have demonstrated the antimicrobial properties of several Common spice oils found that 100 essential oils out of 119 spice oils tested possessed an antagonistic effect to at least 1 of 12 pathogenic fungi and 50 of these samples showed a wide spectrum of activity against all fungi tested **(Maruzze *et al*., 1959).** However, little is known about the antifungal activity of essential oils against fungi which are known to cause damage to structure and decoration

of interior and exterior surface of buildings, their indoor environment and occupants **(Balter *et al.*, 1977).**

Research Objectives

The main aim of this study will be isolation, identification of keratinophilic fungi from the very polluted area of agricultural fields of soil and will check the activity of essential oils extracted from the herbal plants, simultaneously will see the antimycotic studies of essential oils so that a noble drug can be obtained against the dermatophytes which have either vary least side-effect or no side effect for the human beings.

Objectives:

The objectives of proposed work are:-

1. Collection, Isolation, Identification and Maintenance of pure cultures.
2. Screening of keratinase enzyme and check the degradation potential of keratin substrates (nails, hairs, feather, wool) from selected isolates.
3. Optimization of cultural parameters for keratinase production.
4. Collection of different plant parts for the extraction of essential oil and antimycotic studies of essential oils against selected fungi.
5. Determination of MIC (Minimum Inhibitory Concentration) of different essential oil against selected fungi.
6. Studies of different mixture of oils with Standard drugs against selected fungi.

REVIEW OF LITERATURE

Isolate and identify keratinophilic fungi from soils stressed by occurrence of animals, both pets and farm animals. Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors. Such as human and or animal presence, which are of fundamental importance and draws the attention towards the incidence of fungal opportunistic pathogens (*Pseudallescheri aboydii*) found in dog habitat soil. Human pathogens, a geophilic, dermatophyte, *Microsporum, gypseum*was isolated from all six soil samples, stressed by occurrence of a dog. Another soil samples Genus *Chrysosporium has* also occurred namely *Chrysosporium keratinophilum* and *Chrysosporium queensl and icum*. Preliminary results showed that keratinophilic fungi were richly represented in the soils in Slovakia and should pay attention to their occurrence especially in the human environment **(Kacinovo *et al., 2013*).** Fungi found in soil and the variation and prevalence in each area depends on environmental and nutritional conditions. The importance of soil in transmission of diseases, focussing on frequency of dermatophytes and related keratinophilic fungi, potentially pathogenic fungi in various samples collected from two public parks in Ahvaz, South Western Iran. Five hundred samples were collected from different parts of the Zoo and Laleh Parks in Ahvaz. The fungal flora was analysed in different samples for the presence of dermatophytes and keratinophilic fungi by hair baiting technique. The samples were cultured on Sabouraud Dextrose Agar containing chloramphenicol and cyclohexamide, and incubated at 25-27 □C for 4-5 weeks aerobically. Isolates were identified by colony morphology, slide cultures and differentiation tests. Most of the isolated dermatophytes were collected from the soil of the Zoo Park. The most common keratinophilic fungus was *Chrysosporium* and other species were *Trichophyton mentagrophytes, T. verrucosum*, *T. schoenleinii* and *M. gypseum.* The results demonstrated that dermatophytes and related keratinophilic fungi of Ahvaz is somewhat different from that in other parts of Iran. This may be due to the different prevailing climatic conditions **(Zarrin *et al*., 2000).** Keratinophilic fungi are important ecologically and play a significant role in natural degradation of keratinous substrates. Soil that is rich in keratinous materials is most conducive for growth an occurrence of keratinophilic fungi. These are a group of fungi that colonize various keratin substrates and degrade them. Their distribution is variable with the environment and depends on different factors, such as human and or animal presence. Keratin substances which are important natural material, occurring in nature mainly in the form of hairs, wools, feathers, horns,

hooves, nails, skin and other cornfield appendages constitutes natural baits for these fungi**(Khanam *et al*.,2002)**. Keratinophilic fungi include a variety of filamentous fungi, mainly comprising hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a great variety of non dermatophytic filamentous fungi **(Mukesh *et al*., 2010).** Keratinophilic fungi are an ecologically important group of fungi that cycle one of the most abundant and highly stable animal proteins on earth (**Sharma *et al.,* 2003).** The biological function of keratinolytic fungi in the soil is the degradation of keratinized materials such as hides, furs, claws, nails and horns of dead animals. Due to decomposition of keratin substances decay (specialized animals fibre), these fungi are important in soil ecosystem, that is why they are important from the point of element cycle (**Chabasse *et al*., 1994).**These animals that are often asymptomatic carriers of dermatophytes can be important sources of infection(**Ozgur *et al.*,2001)**. A total of 25 keratinophilic fungi including five dermatophytes and closely related species and 20 other fungi were isolated in poultry farm soil from Namakkal, India. The isolated keratinophilic fungi were screened for the extracellular enzymes viz. amylase, protease, cellulase, lipase and keratinase by using plate assay. All of the fungi, with the exception of a non-sporulating fungus, were able to produce at least one of the enzymes and most of the fungi i.e. *Chrysosporium keratinophilum, Aspergillus flavus*, *A. niger, A. terreus, Penicillium citrinum, P. frequentans* and *P. purpurogenum A. versicolor* and *A. niger* synthesized the highest amounts of amylase and cellulose respectively and *P.citrinum* and *Trichodermaviride* produced the highest amount of lipase. Maximum amounts of protease and keratinase were synthesized by *Scopulario psisbrevi caulis*. In addition, *Trichophyton mentagrophytes* and *A. niger* were also potential producer of keratinase. Finally, these keratinophilic fungi were able to produce a wide range of industrial extracellular enzymes. Interestingly, all of the dermatophytes and closely related species produced protease and keratinase **(Anbu *et al.,* 2011).** Keratinolytic fungi are specialized in degradation of which is the main component of keratinous substances (cornfield epidermis, hair, feather, wool, horn, hoof, nails, etc.). These fungi also display lipolytic activity and remove petroleum hydrocarbons from the medium during degradation of proteins (**Kunert *et al*., 2000)**. It was demonstrated in a previous study that inhibition of growth and biomass production of keratinophilic fungi could be used as indicators of leachate toxicity during bioremediation (**Ulfig *et al*.,2000).**The incidence of these fungi also was examined in an acidic petroleum waste lagoon before bioremediation **(Przystas *et al*.,2001).** The goal of this study was to determine what her keratinolytic fungi could be used as indicators of soil petroleum hydrocarbon contamination and bioremediation progress in a bio pile at the

Czechowice refinery site. Feather wastes are generated in large quantities as a by-product of commercial poultry processing. Feathers represent 5-7% of the total dry weight of mature chickens. Feathers are made up primarily of keratin, which is also found in the claws and armour of reptiles and the hooves, horns, hide, hair and nails of mammals **(Tapia *et al*., 2008)**. Keratin is a fibrous and insoluble structural protein extensively cross-linked with disulphide, hydrogen and hydrophobic bonds, resulting in mechanical stability and resistance to common proteolytic enzyme such as pepsin, trypsin and papain. These feathers constitute a sizable waste disposal problem. Several different approaches have been used for disposing of feather waste, including land filling, burning, natural gas production and treatment for animal feed. Most feather waste is land filled or burnt which involves expense and can cause contamination of air, soil and water (**Joshi *et al*.,2007).**Feathers are currently used to produce feather meal through thermal processing, resulting in a low nutritional value product. Feather hydrolysates produced by microbial keratinases have been used as additives for animal feed. In addition, keratin hydrolysates have potential use as organic fertilizers, production of edible films and rare amino acids (**Brandelli *et al.,* 2005)**. There is always a requirement of isolation of enzymes from new sources to meet the industrial and environmental demand. Utilization of these potential keratin degraders will definitely find biotechnological use in various industrial processes involving keratin hydrolysis. It would also solve the waste disposal problem of poultry waste and recycling of keratinacious waste would be beneficial financially and environmentally (**Daroit *et al*., 2010)**. Indian soils contain many more keratinophilic fungi than those presently recorded, and there is need for further taxonomic and ecological studies of this interesting group of organisms **(Sharma *et al.,* 2003)**.In recent years much attention has been paid to studying human pathogenic fungi in their natural habitats. The presence of fungi pathogenic to man in natural environments, especially in soil, has been known, for most of the human-pathogenic fungi, for over a decade. The development of several special techniques for the selective isolation of these fungi from the soil explains the progress made in this field. The method was previously used in isolating keratinophilic hybrids (**Karling 1946)**. Extensive surveys were conducted, using this technique, on the prevalence and distribution of geophilic dermatophytes and related keratinophilic fungi from all over the world. Different plant parts have been widely used for the preparation of folk remedies. Apart from leaves, fruits, and seeds, inflorescence has also been screened for their antimicrobial properties. Green plants because of their vast diversities contain a wide spectrum of plant defence chemicals, most of which make vital contribution to the list of medicines for human even today. At the time of 2500-600 BC, large number of plants has

been reported in Ayurvedic literature which possesses medicinal properties. The Egyptians, Greeks and Romans made use of materials of plant originin human chemotherapy. The green plants for their antimicrobial activity had started since ancient times. Greeks and Romans used the juices of walnut shells against infectious fungal diseases of skin. Mixtures of certain vegetable oils were used by Egyptians for the preservation of meaning from protein decomposing bacteria. In the recent past several attempts were made to screen various plants and plant products for their antifungal activity against the pathogenic fungi, in view of their low phytotoxicity and systemic activity **(Mahadevan 1979).** Several plants contain chemicals, capable of inhibiting the germination and growth of pathogenic fungi. Therefore, in recent years attention has been paid by various researchers towards the screening of some higher plants for their fungi toxic properties. Allelochemicals are substances produced by higher plants that selectively inhibit the growth of microorganisms (virus, bacteria and fungi). Allelopathicn agents encompass a wide array of chemical types, including volatile mono and sesqui terpenoids, phenyl propanoids, quinines, coumarins, flavonoids, tannins and other phenolics and glycosides. Antifungal activity of oilswas studied by against nine human and plant pathogenic fungi (**Garg *et al.,* 1991).**Studied the biological activity of the essential oil andfound effective against keratinophilic fungi *Arthroderma benhamiae*, *Microsporum gypseum*,*Trichophyton mentagrophytes, Ctenomyces serrate* **(Jain *et al.,* 1992)**. Also studied the antifungal activity of Luvunga scadens against some keratinophiilic fungi using filter paper disc agar diffusion technique. The oil showed very good to moderate inhibitory effect against the fungi effect of the extract from eight species of medicinal plants on growth of selected plant pathogenic molds and dermatophytes of all the extracts, the extract obtained from complete fungi static effect on spore germination of all dermatophyte **(Jatisatinr *et al.,* 1996).** The effect of aqueous extracts. All the plants inhibited the growth of dermatophytes investigated a number of essential oils and their constituents for their antimicrobial properties against a series of bacteria and fungi **(Kalemba 1999).** Some of the essential oils were found to possess strongest antimicrobial properties among those tested. **(Okunji *et al.,* 1990)** reported an antifungal spirostanol saponin from fruit pulp of Dracoenamain against six pathogenic fungi and six dermatophytes of which it was strongly active towards *Trichophyton mentagrophytes, T. tonsurans, T.soudanese, T.rubrum,M. audouinii,Cladosporiumspp.*and *Geotrichumspp.* **(Qureshi *et al.,* 1997).** Plant essential oils are potential source of antimicrobials of natural origin. Essential oils and extracts obtained from many plants have recently gained a great popularity and scientific interest. Consumer demand for natural preservatives has increased, whereas the safety aspect of chemical additives has been

questioned. The plant oil has been reported to have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for the development of industrial products. The present review is a compilation of updated information on plant essential oils with antifungal properties (**Tabassum *et al.,* 2013).** Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC) of three medicinal plant essential oils of *Zatariamultiflora, Thymusvulgaris* and *Thymus kotschyanus* on the mycelial growth of four pathogenic fungi including *Pythiumaphani dermatum, Rhizoctoniasolani* (AG4)*Fusarium graminearum* and *Sclerotiniasc lerotiorum.* The rates of growth inhibition were measured after placing active mycelia plugs of each fungus on Petri dishes containing PDA amended with specific concentrations of essential oils and incubated at 28 ± 1 ºC. The data were analyzed using MSTATC and SAS (version 9.1.3) software. The results showed that these essential oils were very effective on the four studied **(Amini *et al.,* 2012).**

Justification/Importance of Study:

The polluted area of agricultural fields of soil is a dynamic medium in which a large number of keratinophilic fungi and non-keratinophilic fungi live in close association. Some keratinophilic fungi are pathogenic to man and animals. Dermatophytic infections are highly common and endemic disease in developing countries due to poverty, improper health facilities, illiteracy and other poorly developed diagnostic techniques also. There is some microorganisms which are resistant to newer or modern antifungal synthetic drug and in some severely immunosuppressed patients. Thus there is a need for an alternative therapy that is safe, cheaper, economical & easily available so wide range of essential oils from plants are still unexplored for their antifungal activity. Hence, the present study aims to isolate & identify the keratinophilic and other fungi from soil sample of U.P and explore new antifungal alternative to synthetic drugs for the treatment of dermatophytic & other fungal diseases. This work will be beneficial for the human health and human economy.

METHODOLOGY

1. Collection of sample

Different animal's habitat and Agricultural field's soil sample will be collected in sterile Container from U.P village and will bring to laboratories for further microbiological analysis.

2. Isolation of fungi

The fungi will be isolated by using different techniques .I.e.

(i) Spread plate method on SDA (Sabouraud Dextrose Agar) media.

(ii) Hair baiting technique (**Vanbreuseghem, 1952)**.

3. Identification of isolated fungus

Identification of fungus will be base on

(i) Morphological (Macroscopic & Microscopic) characterise

(ii) Biochemical characterise

4. Screening of keratinase enzyme

Screening of keratinase enzyme against selected isolates according to **More *et al* (2013).**

5. Check the degradation potential of different keratin substrates

The studies between different isolated fungi to check the degradation potential of different keratin substrate (hair, nails, feathers, wool,) by **Letourneau. (1998)** method.

6. Optimization of cultural parameters for keratinase production (pH, temperature, metals, etc.)

Optimization of cultural parameters for keratinase production according to **More *et al.,* (2013).**

7. Collection of different plants for extraction of essential oil

Different plants will be selected for the extraction of the essential oils by hydro distillation method.

8. Antimycotic/ Comparative studies of mixture of essential oils

Antimycotic studies of essential oils against selected fungi will be seen by Disc Diffusion method.

9. Determination of MIC

MIC of essential oil will be determination by Provine &Hadley method.

10.Studies of mixture oils

Comparative antimycotic studies between single oil and Mixture of oils used are seen by Disc diffusion & MIC method.

Place of Work and Facilities Available:

The place of work will be Jayoti Vidyapeeth Womens University, Jaipur campus. The following facilities will be used.

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